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(19) (CA) **CANADIAN PATENT** (12)

(54) Human Plasma, Plasma Fractions, and Compositions Thereof Substantially Free of Infectious Viruses, Bacteria and Retroviruses

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Invention: HUMAN PLASMA, PLASMA FRACTIONS, AND COMPOSITIONS THEREOF SUBSTANTIALLY FREE OF INFECTIOUS VIRUSES, BACTERIA AND RETROVIRUSES

Abstract of the Disclosure

There is disclosed a method for treating human plasma, plasma fractions and products thereof to render the same substantially free of infectious viruses, bacteria and retroviruses, particularly the retroviruses isolated from patients having the Acquired Immune Deficiency Syndrome (AIDS) by lyophilizing the plasma, plasma fractions or products thereof and then heating the lyophilized material at a temperature of about 60° C to 90° C for about 10 hours to 120 hours.

SPECIFICATION
BACKGROUND OF THE INVENTION

Field of the Invention: This invention relates to a method for heat treating human plasma, plasma fractions and compositions thereof to render them substantially free of infectious viruses, bacteria and retroviruses.

Description of the Prior Art: Many useful blood fractions and blood proteins are obtained from human blood plasma by fractionation according to known techniques.

Among such known techniques, to name but a few representative examples, there may be mentioned the alcohol fractionation method of Cohn et al, U.S. Patent 2,390,074 (1945) and the Journal of the American Chemical Society, 68, 459 (1946); polyethylene glycol fractionation methods of Polson, U.S. Patent 3,415,804, Shanbrom et al, U.S. Patent 3,631,018 (Factor VIII), Schwarz et al, U.S. Patent 4,404,131, and the polyethylene glycol/glycine method of Fekete et al, U.S. Patents 3,682,881 and Re.29,698; the glycine fractionation method of Blomback et al, U.S. Patent 4,348,315; the aluminum hydroxide treatment of an aqueous solution of cryoprecipitate with aluminum hydroxide followed by ultrafiltration and, optionally, glycine treatment of Mitra et al, U.S. Patent 4,386,068; the isolation and purification of Factor IX disclosed in Wada et al, U.S. Patent 3,717,708, and Mitra et al, U.S. Patents 4,361,510 and 4,404,132; the isolation and purification of fibronectin disclosed in Wallace et al, U.S. Patent 4,455,300; the preparation of antithrombin-III disclosed in Jordan, U.S. Patent 4,386,025; and the preparation of alpha-1 proteinase inhibitor disclosed in Coan et al, U.S. Patents 4,379,087 and 4,439,358.

Therapeutic use of such plasma proteins and compositions thereof to treat various disorders has been compromised due

to the risk of contracting virus infection, particularly hepatitis virus infection. Thus, numerous techniques to reduce or eliminate infectious microorganisms and to render plasma protein compositions non-viral infective have been reported.

For example, the preparations, in wet or dry state (that is, as the liquid concentrate itself or freeze-dried), may be heated at temperatures of about 60 to 85° C for a period of several minutes to several days as may be required, generally in the presence of a heat stabilizing agent. Suitable stabilizing agents include nonpolar anions with molecular weights greater than 80, sugars, reduced sugars, and amino acids.

Examples of suitable nonpolar anions include salts of carboxylates, hydroxycarboxylates and amino acids such as a sodium or potassium caprylate, caprate, oleate, laurate, valerate, acetylphenylalaninate, acetyleucinate, and acetyltryptophanate. Examples of suitable sugars include glucose, sucrose and maltose to name but a few, and examples of suitable reduced sugars include erythritol and mannitol. Examples of suitable amino acids include lysine, glysine, proline and glutamic acid to name but a few.

By way of example without limitation, suitable conventional known processes to reduce or eliminate infectious micro-organisms and render the preparations non-viral infective include those disclosed in U.S. Patents 3,041,242, 3,057,781, 3,227,626, 4,061,735, 4,137,307, 4,297,344, 2,705,230, 2,897,123, 3,284,301, 3,454,929, 4,379,085, 4,370,264, 4,440,679 and 4,424,206, and European Patent Publications 0058993, 0077870 and 0094611, and in references disclosed in the patents.

Rubinstein, U.S. Patent 4,456,590 and EP Patent Application Publication 0,096,611, discloses the heat treatment of dry,

for instance, lyophilized, plasma compositions containing Factors VIII and IX at a temperature of at least about 60° C for a time sufficient to render hepatitis virus present in the composition non-infectious.

Since 1978, a disease syndrome has occurred which involves a progressive reduction in cellular immunity leading to the onset of opportunistic infections and cancers, particularly Kaposi's sarcoma and B cell lymphomas. This Acquired Immune Deficiency Syndrome (AIDS) has been observed in the United States primarily in homosexual and bisexual men and lV drug abusers. It has also been found in recipients of blood transfusions, infants of individuals from one of the risk groups, hemophiliacs, and individuals from Haiti and Central Africa. Attempts have been made to isolate the infectious agent responsible for this disease; the leading candidates include herpes (cytomegalovirus) and retroviruses. Because AIDS has occurred in hemophiliacs who have been treated with plasma products, for example, Factor VIII and Factor IX concentrates, the infectious etiologic agent can be assumed to be present in these preparations. Therefore, one characteristic of the agent responsible for AIDS would be its resistance to procedures employed in the concentration and lyophilization of Factor VIII, Factor IX and other plasma products.

Several suspect candidates for the infectious etiologic agent causing the Acquired Immune Deficiency Syndrome have recently been proposed by Jay A. Levy et al, Science, 225, 840 (1984) (ARV), M. Popovic et al, Science, 224, 497 (1984) (HTLV-III), and F. Barré-Sinoussi et al, Science, 220, 868 (1983) and Lancet, pages 753 - 757 (April, 1984) (LAV). These suspect agents are all retroviruses isolated from AIDS patients. The information now available concerning properties of retroviruses demonstrates much diversity among those retroviruses which have been studied in response to exposure to the conditions used in processes

to fractionate plasma and to inactivate viruses and bacteria.

Levy et al, Lancet, pages 722 - 723 (September, 1984) discloses that, upon heating lyophilized Factor VIII concentrate containing a mouse xenotropic type C retrovirus at 68° C for 12 - 48 hours, the infectious agent was still present. However, upon heating the same sample at 68° C for 72 hours, the retrovirus was completely inactivated.

Although the mouse retrovirus present in the dry Factor VIII concentrate was shown to be inactivated by heat treatment at 68° C and 72 hours, the effect of heat treatment in the dry state on the suspect agents for human AIDS has not heretofore been demonstrated.

DESCRIPTION OF THE INVENTION

This invention is based on the discovery that the suspect infectious etiologic agents causing Acquired Immune Deficiency Syndrome (AIDS) in humans can be inactivated by heating a lyophilized composition containing human plasma or plasma fractions or products thereof at temperatures and time periods in the range of about 60 - 90° C for about 10 to 120 hours, preferably about 60 - 80° C for about 24 to 96 hours, more preferably about 65 - 75° C for about 96 hours, and most preferably about 68° C for about 72 hours.

Preferably, the composition comprises a human plasma fraction, or product thereof, consisting essentially of at least one therapeutically active plasma protein selected from Factor VIII, Factor IX, fibronectin, antithrombin-III and alpha-1 proteinase inhibitor. More preferably, the composition contains one of Factor VIII or Factor IX. Most preferably, the composition contains Factor VIII. The

composition used in the method according to the invention can be produced by an of the well-known techniques.

Thus in particular a heat stabilizing agent is added to the composition prior to the lyophilization. Suitable stabilizing agents include nonpolar anions with molecular weights greater than 80, sugars, reduced sugars and amino acids.

Examples of suitable nonpolar anions include salts of carboxylates, hydroxycarboxylates and amino acids such as a sodium or potassium caprylate, caprate, oleate, laurate, valerate, acetylphenylalaninate, acetyleucinate and acetyltryptophanate. Examples of suitable sugars include glucose, sucrose and maltose to name but a few, and examples of suitable reduced sugars include erythritol and mannitol. Examples of suitable amino acids include lysine, glysine, proline and glutamic acid to name but a few.

Generally it is appropriate to select the temperature and time of heating within the defined range in accordance with experimental data inactivation of at least 10^4 particles of AIDS-associated retrovirus.

The following examples illustrate but a few embodiments of the present invention and are not to be construed as limiting in scope. All parts and percentages are by weight and all temperatures are in degrees Celsius unless otherwise indicated.

EXAMPLE 1

The lymphocytopathic retrovirus called "AIDS-related Virus" (ARV), which was recently isolated as reported in Science, 225, 840 (1984) was added to samples of AHF concentrate produced from normal human plasma. The resulting mixtures were freeze-dried and then heated for 48 to 72 hours. The presence of ARV was measured after culturing and concentrates in peripheral mononuclear cells and determining activity of the enzyme, reverse transcriptase (RT). The results showed that a high level of RT was detected in duplicate samples taken at 0 time ($0.5 - 1.5 \times 10^6$ CPM) whereas samples taken after heating at 48 hours and d72 hours showed no significant RT activity. These results indicate that the heat treatment process used inactivated the ARV.

EXAMPLE 2

In this experiment, the samples of AHF concentrate as in Example 1 were inoculated with a different retrovirus, the LAV retrovirus as disclosed in Barré-Sinoussi et al, Science, 220, 868 (1983) and Lancet, pages 753 - 757 (April 1984), and the presence of the LAV retrovirus was detected as described in the reference.

The AHF concentrates were lyophilized and heated at 68°C for varying periods of time and inoculated into microculture plates containing human lymphocytes stimulated with Interleukin-2 and phytohemoglutinin. At 3-day intervals, there was removed some inoculum, culture medium and the removed portion was replaced with fresh cell culture growth medium. After 9 days, the inoculum was transferred to a microculture plate coated with antibody to the LAV. Then, a standard ELISA test was performed to determine the presence of infectious particles. The results showed that at 0 time, there was present $10^{4.27}$ infections particles whereas at each period of 24 hours, 48 hours, 60 hours, 72 hours and 96 hours, there was present less than 2 particles. (The limit of detection was 2 particles).

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method for treating a composition selected from human blood plasma, a human blood plasma fraction and a product produced from human blood plasma and a human blood plasma fraction to render said composition substantially free of infectious viruses, bacteria, and the etiologic agent isolated from patients having the disease syndrome, Acquired Immune Deficiency Syndrome (AIDS), which comprises the steps of:

(a) adding to said composition a heat stabilizing agent;

(b) lyophilizing the composition;

(c) heating the lyophilized composition at a temperature of from about 60°C to 90°C for a period of time of about 10 hours to 120 hours; and

(d) selecting the time and temperature of step (c) in accordance with experimental data showing inactivation of at least 10^4 particles of AIDS-associated retrovirus.

2. A method according to claim 1, wherein said composition comprises a human blood plasma fraction consisting essentially of at least one therapeutically active plasma protein selected from Factor VIII, Factor IX, fibronectin, antithrombin-III, and alpha-1 proteinase inhibitor.

3. A method according to claim 1, wherein said composition comprises a product produced from a human blood plasma fraction consisting essentially of at least one therapeutically active plasma protein selected from Factor VIII, Factor IX, fibronectin, antithrombin-III and alpha-1 proteinase inhibitor.

4. A process of claim 1, wherein the heat stabilizing agent is selected from the group consisting of nonpolar anions with molecular weights greater than 80 daltons, sugars, reduced sugars and amino acids.

5. A method according to claim 1, wherein said lyophilized composition is heated at a temperature of from 65°C to 75°C for a period of time of 24 hours to 96 hours.

6. A method according to claim 1, wherein said lyophilized composition is heated at a temperature of about 68°C for about 72 hours.

7. A method according to claim 2, wherein said lyophilized composition is heated at a temperature of about 68°C for about 72 hours.

8. A method according to claim 3, wherein said lyophilized composition is heated at a temperature of about 68°C for about 72 hours.

9. A pharmaceutical preparation comprising a composition produced according to claim 1, 2 or 3, and a pharmaceutically acceptable carrier.

10. A pharmaceutical preparation comprising a composition produced according to claim 4, 5 or 6, and a pharmaceutically acceptable carrier.

11. A pharmaceutical preparation comprising a composition produced according to claim 7 or 8, and a pharmaceutically acceptable carrier.

SUBSTITUTE

REMPLACEMENT

SECTION is not Present

Cette Section est Absente